

Ciliary Hovering in Larval Lancelets (=Amphioxus)

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Larvae of lancelets (=amphioxus) are of special interest because they figure prominently in debates about vertebrate origins (1), can sometimes grow into a giant "amphioxides" form (2, 3), have a puzzling right-left asymmetry (4), and constitute a major zooplankton resource in parts of the Atlantic (5). By using improved methods (6, 7) to culture and observe healthy pre-metamorphic larvae in relatively deep containers, we demonstrated a prominent hovering behavior. The larvae spend most of their time suspended in midwater by metachronal beating of epidermal cilia. The body is usually tilted at an angle such that the anterior end and ventral side are oriented towards the water surface. This posture is maintained in the dark and in the light, although there is directional photosensitivity. Hovering may help account for the giant "amphioxides" and may be related to the curious asymmetry of the larval body.

We raised developing lancelets (*Branchiostoma floridae*) in the laboratory (6, 7) in 8-cm diameter containers filled to a depth of 6 cm with seawater, and we used a television camera fitted with a macro lens (8) to record behavior at room temperature (23°C). Locomotion of the embryos (hatching to 2 days after fertilization) was by the spiral, ciliary swimming that has been described previously (9). In contrast, during the month-long stage of the pre-metamorphic larva, the behavior differs markedly from most previously published accounts, which were often based on gradually starving animals maintained in very shallow dishes. We found that the larvae cultured in deeper water spent most of their time hovering almost motionlessly in midwater at various depths in the culture vessel. The body of each hovering larva was oriented at an angle of about 60° from horizontal, with the anterior end and ventral side oriented toward the water surface (Fig. 1).

The motive force for larval hovering was the beating of epidermal cilia in metachronal waves that pass down the body from anterior to posterior at about 0.3 mm/s (Fig. 2). Brief exposure to 0.1% glutaraldehyde in seawater arrested ciliary beating, and the larvae sank, usually anterior first, at about 0.25 mm/s, close to values previously found for larvae of another lancelet species (10). This sinking indicates that the larvae do not use gas bubbles or a high lipid content to remain suspended. Drag was calculated by the formula for the low Reynolds number drag of a cylinder moving parallel to its long axis (11). By equating this drag to the forces (buoyant and gravitational) acting on the larvae, we estimated that approximately 1.4×10^{-9} Newtons were required for ciliary hovering.

The hovering larvae maintain their characteristic slanted posture in the dark and in moderate light. They do, however, show some directional photosensitivity: as seen from above, they will slowly orient themselves with their heads away from an eccentrically placed light source (Fig. 3). Because complete orientation requires about 20 min, it is difficult to understand what, if any, function this could have in the natural environment. Significantly, however, the phenomenon indicates that lancelet larvae have directional photoreceptors. As recently proposed (12), such receptors could well be the neurons associated with the pigment spot at the anterior end of the cerebral vesicle. When the head of a hovering larva is oriented directly away from an eccentric light source, the pigment spot would provide maximal shading to the putative photoreceptive cilia of the anterior neurons.

Our results vindicate Willey (13), who clearly described larval hovering in *Branchiostoma lanceolatum*, but whose observations were never repeated and were strongly doubted by some (10, 14). In our cultures, the few larvae that were not hovering swam for a few seconds at infrequent intervals by muscular undulation or occasionally crawled on the bottom of the container by means of their

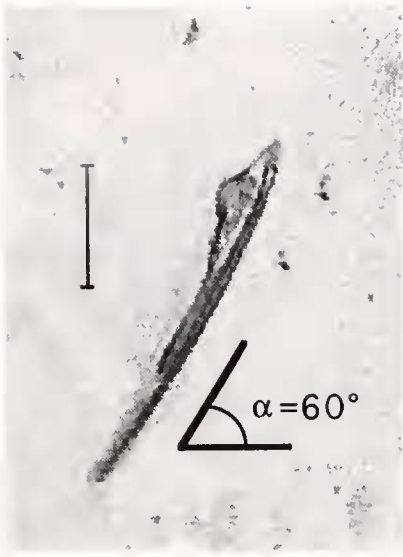


Figure 1. Lateral view (from video) of a hovering, 4-day-old lancelet larva, illuminated by white light. The body axis is oriented 60° from the horizontal and the anterior end and ventral surface is uppermost, directed towards the surface. The long axis of the body is at an angle (α) of about 60° from horizontal; for a random sample of 4-day-old larvae ($n = 115$) the mean angle was $61^\circ (\pm 1 \text{ SD} = 6.7)$. Scale bar, 0.5 mm.

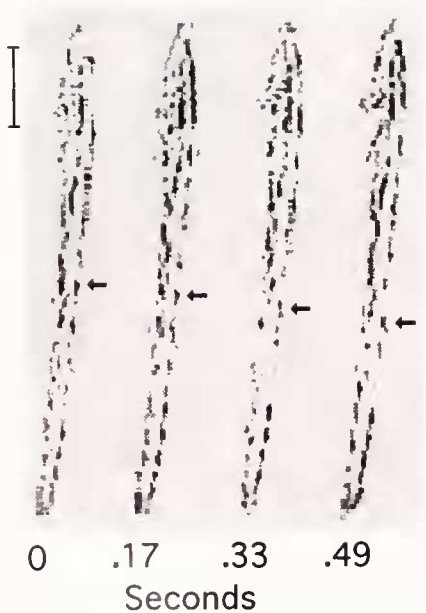


Figure 2. Lateral views of a hovering 4-day-old larva from video (30 frames/s) taken at 5-frame intervals. Animals were observed under darkfield laser Schlieren illumination (25) to visualize the beating of the epidermal cilia. The arrowheads follow the movement of one of a series of metachronal waves moving down the body from the anterior (top) to the posterior (bottom) at approximately 0.3 mm/s. Scale bar, 0.25 mm.

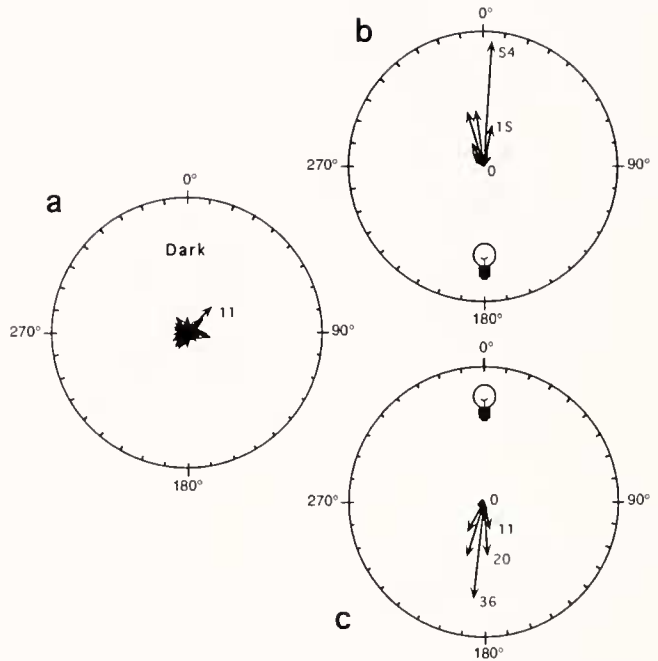


Figure 3. Orientation of 4-day-old hovering larvae, as viewed from above, in the presence and absence of an incandescent light source (intensity approximately 2.6 log Lum/m^2 , positioned 50 cm from the observation vessel). The anterior ends of the larvae point in the direction of the arrowheads, while the length of the arrow shaft shows the number of larvae in each 10° sector. The number of larvae in some sectors is indicated. Orientations of a group of larvae ($n = 125$): (a) after 3 h of darkness; (b) after 20-min exposure to a light source originating from one side; (c) 20 min after moving the light to the opposite side of the observation vessel.

epidermal cilia. Most previous studies of larval lancelets were under conditions (very shallow culture containers and insufficient food) that did not permit hovering, but mainly favored ciliary crawling interspersed with brief episodes of undulatory swimming. Some studies (14, 15) even used such unsanitary culture conditions that the larvae became heavily infected with bacteria, moribund, and attached artifactually to the substratum.

Pre-metamorphic larval lancelets are not infrequently captured in the plankton (5, 10, 16), sometimes even far above the ocean bottom, although it has never been determined how they remain in midwater. Plankton ecologists usually imply that such larvae use muscular undulation continuously or sporadically to remain in the water column (10, 14, 17); however, we prefer the minority view (9, 13) that lancelet larvae under field conditions remain suspended chiefly by the beating of their epidermal cilia. In comparison to undulatory swimming, ciliary hovering probably conserves energy, favors continuous filter feeding, is less attractive to predators, and permits the larvae to attain a relatively large planktonic size.

Lancelet larvae lose most of their cilia at metamorphosis (7). However, the "amphioxides" larvae (2, 3, 18, 19) delay

metamorphosis and evidently the loss of cilia. Such larvae could use energy-efficient ciliary hovering to remain continuously in the plankton where they can then attain lengths up to 13.8 mm. In contrast, the post-metamorphic lancelets that are occasionally (and temporarily) present in the plankton (20) must remain in the water column entirely by muscular undulation, which is probably less efficient than ciliary hovering.

The ciliary hovering of lancelet larvae could well be related to their curious asymmetry, which has been explained by some as inherited from an ancestor (21, 22), but by others as a larval adaptation (23, 24). We favor the latter view and suggest that larval hovering co-evolved with a laterally compressed body that, by optimizing the surface-to-volume ratio, increased the effectiveness of the ciliary propulsion. Such lateral compression may in turn be related to the asymmetric displacement of the larval mouth to one of the broad body surfaces [as previously suggested by Bone (2)] simply to ensure an opening large enough to permit effective filter feeding.

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